Host Factors Permissive to Therapeutic Liver Repopulation

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Therapeutic liver repopulation is a process in which transplanted donor cells extensively replace the hepatocellular compartment of damaged host liver. Hepatocyte replacement levels of >90% have been achieved in multiple animal models either by conditioning with exogenous agents or by taking advantage of liver transgenes. In *Mus musculus*, a genetically tractable organism, knockout of the fumarylacetoacetate hydrolase gene (*Fah*) is the most robust of these models. Fah+ donor hepatocytes begin to proliferate within one week after transplantation, resulting in near-complete repopulation by 6 weeks. In order to understand the basis of this phenomenon, rates cell division and cell death were compared in donor and host hepatocytes. Unexpectedly, the *Fah* mutant host hepatocytes but not the donor cells displayed a profound cell cycle arrest together with apoptosis resistance. This combination explains why donor cells expand differentially and why the animals survive the repopulation period. The apoptosis resistance in host hepatocytes provides a window of time during which they can support the animal metabolically while repopulation is happening.

In order to understand the molecular basis for both cell cycle arrest and apoptosis resistance, microarray studies were performed. The cell cycle regulatory protein p21 was found to be strikingly elevated, providing a potential explanation for both the growth arrest and cell death resistance phenotypes. To test this hypothesis, *Fah/p21* double mutant mice were generated. Interestingly, continuous hepatocyte proliferation was observed in these animals. In addition, *Fah/p21* double mutant cells, but not Fah single mutants underwent apoptosis after injection of the Fas-agonist Jo2. Thus, p21 expression in the Fah mutant hepatocytes is the single dominant factor contributing to both phenotypes which are important for liver repopulation. Accordingly, transplantation of Fah+ hepatocytes into *Fah/p21* double mutant mice resulted in markedly reduced levels of liver repopulation.

To understand the apoptosis resistance phenotype in more detail, molecular events after Jo2 injection were studied. We found that the apoptosis signaling pathway was blocked downstream of caspase-8 activation and upstream of mitochondrial cytochrome C release in *Fah* mutant hepatocytes. It was further determined that the pro-apoptotic Bcl2-like protein Bid remained phosphorylated and was not cleaved despite caspase-8 activation, thereby preventing mitochondrial activation. We propose a model in which chronic stress results in high levels of caspase 8 resistant p-Bid and thereby increases the threshold for apoptotic cell death hepatocytes. These increased levels of p-Bid may be due to the p21-dependent activation of a stress-induced kinase or inhibition of phosphatases or both.

These findings suggest that genetic or pharmacological manipulation of p21 and/or p-Bid in host hepatocytes could provide a host environment suitable for therapeutic repopulation.